UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/823,784	04/14/2004	Karen Uhlmann	3035-101	4952

46002 7590 03/14/2006

JOYCE VON NATZMER
4615 NORTH PARK AVENUE, SUITE 919
CHEVY CHASE, MD 20815

EXAMINER	
SHAW, AMANDA MARIE	

ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 03/14/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/823,784	UHLMANN ET AL.	
	Examiner	Art Unit	
	Amanda M. Shaw	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-32 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-32 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on 17 August 2004 is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☒ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date 3/11/05, 2/21/06.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. On 2/27/2006 a non final first office action was mailed. However, a prelimiary amendment was submitted to the office prior to the mailing of the office action. The preliminary amendment amended claims 1, 5, 7, 9, 16, 20, 22, and 24 and added new claims 28-32. As a result a new office action is issued herein. Accordingly the previous office action is vacated and a new office action is set forth below.

Oath/Declaration

2. The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

The oath or declaration is defective because: on the declaration the applicant is claiming foreign priority benefits under 35 U.S.C. 119 (a)-(d) and 365 (b) using U.S. provisional application 60/462289. A new oath/declaration is required in which priority to provisional application 60/462,289 is claimed under 35 U.S.C. 119(e).

Specification

The specfication is objected to because the specification needs to include a section entitled Brief Description of the Drawings. On page "3" of the specfication the heading saying "The figures show" should be deleted and the title "Brief Description of the Drawings" should be inserted therefor.

See 37 CFR 1.74.

Claim Rejections - 35 USC § 112

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-27 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-27 recite the limitation "said nucleic acid molecule" There is insufficient antecedent basis for this limitation in the claim.

Claims 1, 9, 12 and 24 recite the limitation "said amplified nucleic acid". There is insufficient antecedent basis for this limitation in the claim.

Claim 7 and 22 recite the limitation "said label". There is insufficient antecedent basis for this limitation in the claim.

Claim Rejections - 35 USC § 103

4. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-14, 19-32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gonzalgo et al (U.S. Patent 6251594) in view of Nyren et al (U.S. Patent 6258568).

Regarding Claim 1, Gonzalgo et al teach a method for the detection of the methylation status of a nucleotide at a predetermined position in a nucleic molecule comprising the steps of (a) treating the nucleic acid molecule with an agent suitable for the conversion of said nucleotide if present in (i) methylated form; or (ii) non-methylated form to pair with a nucleotide normally not pairing with said nucleotide prior to conversion; (b) amplifying said nucleic acid molecule. Specifically Gonzalgo et al teach a method for determining the methylation status at cytosine residues. The steps taught by Gonzalgo et al comprise (a) treating genomic DNA with sodium bisulfite to convert unmethylated cytosine residues to uracil residues while leaving any 5-methylcytosine residues unchanged to provide primers specific for the bisulfite-converted genomic sample for top strand or bottom strand methylation analysis (b) performing a PCR amplification procedure using the top strand or bottom strand specific primers (Column 4, lines 1-11).

Gonzalgo et al do not teach a method wherein after the amplification step, real-time sequencing is performed to determine the methylation status at a predetermined nucleotide position in the sample.

However, Nyren et al teach a method wherein real-time sequencing is performed to determine the methylation status at a predetermined nucleotide position in the sample. Specifically Nyren et al teach a real time sequencing method called

pyrosequencing that can be used to identify a base at a predetermined position in a DNA sample using an extension primer, which hybridizes immediately adjacent to the target position. The DNA sample and extension primer are subjected to a polymerase reaction in the presence of a each dNTP separately and the dNTPs will only become incorporated and release pyrophosphate (PPi) if it is complementary to the base in the target position. When the PPi is released a certain amount of light gets released that is equivalent to the amount of incorporated nucleotides. The unincorporated dNTPs get degraded (Column 2, lines 25-43). This method can be used to determine base changes caused by methylation in samples treated with sodium bisulfite because the treatment converts unmethylated cytosine to uracil which gets amplified as thymine. Methylated cytosine remains unchanged and gets amplified as guanine. Therefore if an adenine gets incorporated then we would know that they cytosine was methylated. If a cytosine gets incorporated we would know that the cytosine was unmethylated.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Gonzalgo et al as to have used the method pyrosequencing taught by Nyren et al, in order to achieve the benefits of a method which has a higher throughput at a cost effective rate. Advantages of using pyrosequencing to detect methylation are as follows: the method is suitable for handling of multiple samples in parallel; relatively cost-effective instruments can be envisioned; and the method avoids the use of electrophoresis and thereby the loading of samples and casting of gels so it is faster. Accordingly, one of ordinary skill in the art

would have been motivated to have modified the method of Gonzalgo in order to have achieved these advantages.

Regarding Claim 2, Gonzalgo et al and Nyren et al both teach a method wherein the sample is derived from a tissue, a body fluid, or stool. Specifically Gonzalgo et al teach that genomic DNA should be used and can be obtained from the following sources: cell lines, blood, sputum, stool, urine, cerebrospinal fluid, paraffin-embedded tissues, histological slides and combinations thereof (Column 6, lines 22-27). Specifically Nyren et al teach that the sample DNA may be provided by any desired source of DNA (Column 7, lines 65-68).

Regarding Claim 3, Gonzalgo et al teach a method wherein the sample is derived from a tumor tissue, a neurodegenerative tissue or a tissue affected with another neurological disorder. Specifically the tissues used by Gonzalgo et al were from tumor tissues from colon and bladder cancer (Example 3).

Regarding Claim 4, Gonzalgo et al and Nyren et al both teach that nucleic acid molecule being detected is a DNA or RNA molecule. Gonzalgo et al teach a method based upon DNA methylation (Abstract) and Nyren et al teach a method of sequencing DNA (Abstract).

Regarding Claim 5, Gonzalgo et al and Nyren et al both teach that the amplification step is done LCR or PCR. Gonzalgo et al teaches the amplification step is done by PCR (Column 4, lines 9-10). Nyren et al teach that sample DNA being used can be PCR amplified fragments (Column 7, lines 66-67).

Regarding Claims 6 and 7, in the pyrosequencing method of Nyren et al, one amplification primer is detectably labeled with biotin, avidin, streptavidin or a derivative or a magnetic bead. Specifically Nyren et al teach that immobilization of the amplified DNA may take place as part of PCR amplification itself, as where one or more primers are attached to a support, or alternatively one or more of the PCR primers may carry a functional group permitting subsequent immobilization, eg. a biotin or thiol group (Column 8, lines 22-27).

Regarding Claim 8, Gonzalgo et al teach a method wherein the methylated nucleotide is an adenine, guanine, or cytosine. Specifically the method taught by Gonzalgo et al is for the determination of methylation status at cytosine residues (Abstract).

Regarding Claim 9, in the real time pyrosequncing method of Nyren et al, the method comprises: (a) hybridization of a sequencing primer to said amplified nucleic acid molecule in single-stranded form (Column 2, lines 25-29); (b) addition of a DNA polymerase, a ATP sulfurylase, a luciferase, an apyrase, adenosine-phosphosulfate (APS) and luciferin(Column 6, lines 60-65, Column 7, lines 1-5); (c) sequential addition of all four different dNTPs (Column 2, lines 29-33) (d) detection of a luminescent signal wherein the intensity of the luminescent signal is correlated with the incorporation of a specific nucleotide at a specific position in the nucleic acid molecule (Column 2, lines 33-36) wherein the detection of the signal is indicative of a the methylation status.

Regarding Claim 10, in the pyrosequencing method of Nyren et al, Nyren teaches a method comprising quantifying the methylated nucleotides. Specifically Nyren et al teach that the observed signals (amount of light given off when PPi is released) is proportional to the amount of nucleotides incorporated into the sample (Column 18, lines 66-67 and Column 19, lines 1-2).

Regarding Claim 11, Gonzalgo et al teach that a suitable agent for the conversion of a nucleotide to pair with a nucleotide normally not pairing with said nucleotide is a bisulfite, preferably sodium bisulfite. Specifically Gonzalgo et al teach a method of treating genomic DNA with sodium bisulfite to convert unmethylated cytosine residues to uracil residues while leaving any 5-methylcytosine residues unchanged (Column 4, lines 3-9).

Regarding Claim 12, Gonzalgo et al teach a method for the diagnosis of a pathological condition or the predisposition for a pathological condition comprising detection of the methylation status of a nucleotide at a predetermined position in a nucleic acid molecule comprising the steps mentioned above. Gonzalgo et al teach that the method can be used to diagnose cancer (Abstract).

Regarding Claim 13, Gonzalgo et al teach a method wherein said pathological condition is cancer, a neurodegenerative disease or another neurological disorder. Gonzalgo et al teach that the method can be used to diagnose cancer (Abstract).

Regarding Claim 14, Gonzalgo et al teach a method wherein the cancer is a primary tumor, a metastasis or a residual tumor. The DNA samples analyzed by

Gonzalgo et al were primary melanoma tumor tissue samples and primary bladder tumor tissue samples (Column 5, lines 35-37).

Regarding Claim 19, Gonzalgo et al and Nyren et al both teach that nucleic acid molecule being detected is a DNA or RNA molecule. Gonzalgo et al teach a method based upon DNA methylation (Abstract) and Nyren et al teach a method of sequencing DNA (Abstract).

Regarding Claim 20, Gonzalgo et al and Nyren et al both teach that the amplification step is done LCR or PCR. Gonzalgo et al teaches the amplification step is done by PCR (Column 4, lines 9-10). Nyren et al teach that sample DNA being used can be PCR amplified fragments (Column 7, lines 66-67).

Regarding Claims 21 and 22, in the real time pyrosequencing method of Nyren et al, one amplification primer is detectably labeled with biotin, avidin, streptavidin or a derivative or a magnetic bead. Specifically Nyren et al teach that immobilisation of the amplified DNA may take place as part of PCR amplification itself, as where one or more primers are attached to a support, or alternatively one or more of the PCR primers may carry a functional group permitting subsequent immobilisation, eg. a biotin or thiol group (Column 8, lines 22-27).

Regarding Claim 23, Gonzalgo et al teach a method wherein the methylated nucleotide is an adenine, guanine, or cytosine. Specifically the method taught by Gonzalgo et al is for the determination of methylation status at cytosine residues (Abstract).

Regarding Claim 24, the real time pyrosequencing method of Nyren et al comprises: (a) hybridization of a sequencing primer to said amplified nucleic acid molecule in single-stranded form (Column 2, lines 25-29); (b) addition of a DNA polymerase, a ATP sulfurylase, a luciferase, an apyrase, adenosine-phosphosulfate (APS) and luciferin(Column 6, lines 60-65, Column 7, lines 1-5); (c) sequential addition of all four different dNTPs (Column 2, lines 29-33) (d) detection of a luminescent signal wherein the intensity of the luminescent signal is correlated with the incorporation of a specific nucleotide at a specific position in the nucleic acid molecule (Column 2, lines 33-36) wherein the detection of the signal is indicative of a the methylation status.

Regarding Claim 25, Gonzalgo doesn't specifically teach quantifying the methylated nucleotides, but Nyren does. Nyren et al teaches a method comprising quantifying the methylated nucleotides. Specifically Nyren et al teach that the observed signals (amount of light given off when PPi is released) is proportional to the amount of nucleotides incorporated into the sample (Column 18, lines 66-67 and Column 19, lines 1-2). It would have been obvious to one of ordinary skill in the art at the time the invention was made to have quantified the methylated nucleotides to determine the methylation status of the nucleotides which can be used to diagnose disease.

Regarding Claim 26, Gonzalgo et al teach that a suitable agent for the conversion of a nucleotide to pair with a nucleotide normally not pairing with said nucleotide is a bisulfite, preferably sodium bisulfite. Specifically Gonzalgo et al teach a method of treating genomic DNA with sodium bisulfite to convert unmethylated cytosine

residues to uracil residues while leaving any 5-methylcytosine residues unchanged (Column 4, lines 3-9).

Regarding Claim 27, Nyren et al teach that pyrosequencing is a high throughput method. Specifically Nyren states that their method overcomes the need for a high throughput way of sequencing DNA (Column 2, lines 11-22).

Regarding Claims 28-29, Gonzalgo et al teach a method wherein the sample is derived from a tissue, a body fluid, or stool. Specifically Gonzalgo et al teach that genomic DNA should be used and can be obtained from the following sources: cell lines, blood, sputum, stool, urine, cerebrospinal fluid, paraffin-embedded tissues, histological slides and combinations thereof (Column 6, lines 22-27).

Regarding Claim 30, Gonzalgo et al teach a method wherein said nucleotide is a cytosine and is part of one of the following sequences: CpG, CpNpG or CpNpN. Specifically Gonzalgo et al teach a cancer diagnostic method based upon DNA methylation differences at specific CpG sites (Abstract). The method taught by Gonzalgo et al is used to determine DNA methylation patterns at cytosine sites (Column 3, lines 65-67).

Regarding Claim 31, Gonzalgo et al teach a method wherein the methylation status of more than one predetermined nucleotide is detected and a number of samples are analyzed at the same time. Specifically Gonzalgo et al teach a method wherein Ms-SNuPE primers are designed to anneal to and terminate immediately 5' of a desired codon in the CpG target site (Column 8, lines 41-44). In Example 1, three Ms-SNuPE

primers were used to anneal to and terminate immediately 5' of three different target sites. The methods of Gonzalgo are also able to examine a large number of samples at once. Gonzalgo et al teach that the after the Ms-SNuPE primer extension reactions are performed, the products are transferred to nylon membranes which allows for a large number of samples to be analyzed simultaneously in a high density format (Column 5, lines 56-60).

Regarding Claim 32, Gonzalgo et al teach a method of determining the amount of a pairing partner in an amplification product. Specifically Gonzalgo et al teach a method wherein samples are treated with bisulfite, amplified, and the amplification products are detected using ^{32}P or a fluorescent probe (Column 7, lines 58-63). This technique is used to determine the relative amount of methylation at each CpG site.

5. Claims 15, 16, and 18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gonzalgo et al (U.S. Patent 6251594) in view of Nyren et al (U.S. Patent 6258568) and in further view of Herman (U.S. Patent 5786146).

The teachings of Gonzalgo et al and Nyren et al are presented above. The combined references do not teach that the primary tumor is a glioma selected from the group comprising: astrocytoma, oligodendroglioma, an oligoastrocytoma, a glioblastoma, a pilocytic astrocytoma. The combined references also do not teach that the neurological disorder is selected from the group comprising: Prader-Willi-Syndrome, Angelman-Syndrome, Fragile-X-Syndrome, or ATR-X-Syndrome.

However, Herman et al teaches that the detection of methylated CpG containing nucleic acid is indicative of several disorders. Such disorders include but are not limited to low grade astrocytoma, anaplastic astrocytoma, glioblastoma, medulloblastoma, colon cancer, lung cancer, renal cancer, leukemia, breast cancer, prostate cancer, endometrial cancer and neuroblastoma. Identification of methylated CpG status is also useful for detection and diagnosis of genomic imprinting, fragile X syndrome and X-chromosome inactivation.

Accordingly it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the method that Gonzalgo et al used to diagnose primary tumors, to also diagnose gliomas and neurological disorders. It was well known in the art at the time the invention was made that the detection of methylated sequences is indicative of certain gliomas and neurological disorders. Accordingly, one of ordinary skill in the art would have been motivated to have modified the method of Gonzalgo in order to have achieved the advantage of being able to diagnose these additional diseases.

6. Claim 17 is rejected under 35 U.S.C. 103(a) as being unpatentable over Gonzalgo et al (U.S. Patent 6251594) in view of Nyren et al (U.S. Patent 6258568) and in further view of Feinberg (Pub No. US 2003/0232351).

The teachings of Gonzalgo et al and Nyren et al are presented above. The combined references do not teach a method used to diagnose neurodegenerative diseases such as Alzheimer's disease, Parkinson disease, Huntington disease, or Rett-Syndrome.

However, Feinberg teaches a method of determining a disease state in a subject by determining DNA methylation status. Although the disease state is often cancer, the methods taught by Feinberg also include Alzheimer's disease and Parkinsons disease (Paragraph 0029).

Accordingly it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the method that Gonzalgo et al used to diagnose primary tumors, to also diagnose neurodegenerative diseases. It was well known in the art at the time the invention was made that the detection of methylated sequences is indicative of certain neurodegenerative diseases. Accordingly, one of ordinary skill in the art would have been motivated to have modified the method of Gonzalgo in order to have achieved the advantage of being able to diagnose these additional diseases.

Conclusion

7. No Claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Amanda M. Shaw whose telephone number is (571) 272-8668. The examiner can normally be reached on Mon-Fri 7:30 TO 4:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached at 571-272-0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Amanda M. Shaw
Examiner
Art Unit 1634
March 8, 2006

CARLA J. MYERS
PRIMARY EXAMINER